The HLA-class I Restricted CTL Response in HIV-1 Infection: Identification of Optimal Epitopes

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I. Introduction

HIV-1 infection is associated with a vigorous HLA class I restricted cytotoxic T lymphocyte (CTL)response[reviewedin 1]. Byanalogy to anumber ofanimalmodels ofviral infection, theseCTL arelikelyto playan importantrole asahostdefense in infectedpersonsandare likelyto beanimportant component of an effective AIDS vaccine. A large number of laboratories have now contributed to the identification of peptides containing HIV-1 CTL epitopes. In terms of class I-restricted CTL epitopes, studies have shown that these peptides are typically 8–11 amino acids in length and conform to certain motifs for different class I molecules [2]. A comprehensive list of peptides reported to contain putative CTL epitopes has been included in the data base. Many of these have not been further defined as to the optimal peptides required for recognition, or the restricting class I molecule. In addition, a number of peptides have been reported which do not appear to contain regions corresponding to binding motifs for the restricting class I molecules. Therefore some of these peptides require further investigation to confirmtheir immunogenicity and their HLArestriction. Weattempt hereto identify those HIV derived epitopes for which the optimal peptide has been precisely identified and for which the restricting molecule is well defined.

II. Characteristics of viral CTL epitopes

MHC class I restricted CTL recognize processed viral peptides that are presented in an antigen binding site on the class I molecule formed between two alpha helices, with the floor of the groove formed by a beta sheet [2,3]. A number of methods have been used to determine the identity of the presented peptides. The most precise definition has come from elution of such peptides from class I molecules, revealing consistent motifs shared by peptides presented by the same class I molecule. The bindingmotifsarecharacterizedbyanchorresidueswhichserveascontactsitesbetweenthepeptideand specific pockets in the class I binding groove [4]. The amino and carboxy termini of epitopic peptides fit into the A and F pockets of this groove, respectively, due to hydrogen bonding. The amino terminal anchors of the peptides are variable in position and number, whereas the carboxy anchor is always at the C-terminus, with the side chain pointing directly into the bottom of the F pocket. Although there is considerable heterogeneity among amino terminal anchor residues, the F pocket appears to place more restrictions on the amino acids it will accommodate, such that either leucine, isoleucine, arginine, tyrosine, valine or phenylalanine is at the C-terminus of over 95% of known epitopes [5]. Motif predictions for antigenic peptides have now been generated for a large number of HLA class I molecules, although the majority of class I epitope motifs are yet to be defined.

III. HIV-1 CTL epitopes

HIV-derived, HLA class I restricted CTL epitopes have been defined by a number of different strategies, not onlyin terms oftheeffector cells and thetargetcells used, but alsoin termsofthe method of antigen presentation. Effector cells have consisted of freshly isolated bulk PBMC, CD8-positive cell lines stimulated in vitro with either whole virus, recombinant antigen, or peptide containing a CTL epitope, and CTL clones obtained by limiting dilution [reviewed in 1]. Each of these methods has led to reliable definition of certain epitopes. In terms of antigen presentation, the majority of CTL epitopes have been defined using a two part strategy consisting of recombinant protein expression,

usually with vaccinia expression systems, followed by the use of short synthetic HIV-1 peptides to sensitize autologous B cell lines for lysis. Optimal epitopes have usually been defined as that peptide which results in maximal lysis at the lowest peptide concentration.

With the description of HLA allele specific binding motifs, a new method to identify peptides binding to a certain class I molecule became possible. Based on the anchor residues described for various HLA alleles, viral protein sequences can be screened for the presence of such anchor residues [4,6]. These peptides are then synthesized and tested either in cellular or cell free assays for their capacity to bind to the selected class I molecule, and the ability of peptides thus defined to serve as a target for CTL in natural infection is then determined, typically by in vitro stimulation of PBMC with the peptide in question. This "predictive" approach can also facilitate the identification of optimal peptides when larger peptides containing CTL epitopes have been defined, by identifying regions in the larger peptide which conform to the proposed motif. A more precise method to define optimal CTL epitopes is represented by peptide elution studies, although only few such studies have been performed in the HIV-1 system [7]. Infected cells are expanded to large numbers and their HLA class I molecules are isolated. Peptides within the class I binding groove can then be released from the class I molecule with trifluoroacetic acid and analyzed by HPLC. The relevant peaks in the HPLC profile can besequencedandrepresentepitopesproducedwithintheinfectedcell[8,9]. Becauseepitopesidentified in this manner have been identical to optimal peptides identified by performing peptide titrations with truncated synthetic peptides, the latter system appears to be a reasonable alternative [7].

Since the first description of HIV specific CTL 8 years ago, an increasing number of CTL epitopes have been identified by using one of the above described methods. However, the reliability of the described optimal peptides depends strongly on the method(s) used. The complete list of HIV derived peptides containing CTL epitopes is included in the data base. Because the same rigid criteria have not been applied for all of the peptides containing HIV-1 CTL epitopes published to date, we have compiled here a list of those peptides for which there are reasonably good data regarding the HLA restriction and optimal peptide (Table 1). From almost 200 HIV peptide sequences reported to contain class I restricted CTL epitopes, only peptides were selected which1) have been truncated to the minimal peptide required for sensitization, 2) where the optimal epitope has been identified based on motif prediction, dilution curves or peptide elution and 3) where clear definition of the HLA restriction element has been shown. The listed peptides do not necessarily fulfill the binding motifs given in Table 1,reflectingthelimited efficiencyofthebindingmotifbasedpredictiveapproach. Thepeptidesreported to contain CTL epitopes but which are not included in Table 1 lack in most cases data on the minimal epitope. Furthermore, there areabout 20additional peptides forwhichthe HLA restrictionhas not been clearly defined thus far.

Table 1 Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-A2				2 C L V I L	Falk 91	9
	p17	77–2	LAI	SLYNTVATL	Johnson 91, Parker 92,94	23,24,25
	RT	346–2	LAI	VIYQYMDDL	T. Harrer	U
	RT	476–2	LAI	ILKEPVHGV	Walker 89, Tsomides 91	26,27
	gp41	814–2	LAI	SLLNATDIAV	Dupuis 95	28
	nef	190–2	LAI	AFHHVAREL	Hadida 95	14

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-A3.1				23 C IF K L Y	Di Brino 93	29
	p17	18–2	LAI	KIRLRPGGK	T. Harrer	S
	p17	20–2	LAI	RLRPGGKKK	B. Culmann	PC
	gp120	37–2	LAI	TVYYGVPVWK	Johnson 94 b	30
	gp41	775–2	LAI	RLRDLLLIVTR	Takahashi 91	31
	nef	73–2	LAI	QVPLRPMTYK	Koenig 90, Culmann 91	32,33
HLA-A11				2 C I K L	Zhang 93	34
	p17	84–2	LAI	TLYCVHQRI	T. Harrer	U
	RT	325–2	LAI	AIFQSSMTK	Johnson 94 a, Zhang 93	13,34
	RT	508–2	LAI	IYQEPFKNLK	B. Culmann	PC
	nef	73–2	LAI	PLRPMTYK	Culmann 91	33
	nef	84–2	LAI	AVDLSHFLK	Culmann 91	33
HLA-A24						
	gp120	53–2	LAI	LFCASDAKAY	Lieberman 92, Shankar 95	35,36
	gp41	591–2	LAI	YLKDQQLL	Dai 92	37
HLA-A25						
	p24	145–2	LAI	QAISPRTLNAW	I. Kurane, K. West	PC
	p24	203–2	LAI	ETINEEAAEW	P. Klenerman	IP
HLA-A31						
	gp41	775–2	LAI	RLRDLLLIVTR	Safrit 94 a, Safrit 94 b	38,39
HLA-A32						
	RT	559–2	LAI	PIQKETWETW	T. Harrer	S
	gp120	419–2	HXB2	RIKQIINMW	T. Harrer	S
HLA-B7				123 C APR L	Engelhard 93	40
	gp120	303–2	LAI	RPNNNTRKSI	J. Safrit and R.A. Koup	PC
	gp41	848–2	LAI	IPRRIRQGL	B. Wilkens	U
	nef	128–2	LAI	TPGPGVRYPL	Culmann 94	41, PC
HLA-B8				3 5 C K K I	Hill 92, Sutton 93	42,43
	p17	24–2	LAI	GGKKKYKL	Rowland-Jones 93, S. Reid	44, U
	p24	259–2	LAI	GEIYKRWII	Sutton 93	43
	p24	329–2	LAI	DCKTILKAL	Sutton 93	43
	RT	185–2	LAI	GPKVKQWPL	Walker 89, Sutton 93	26,43
	gp41	591–2	LAI	YLKDQQLL	Johnson 92, Shankar 95	45,36
	nef	90–2	LAI	FLKEKGGL	Culmann 94, P. Gould	41, PC

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-B14				23 56 C RL RI L KY HL F	Di Brino 94	46
	p24	183–2	LAI	DLNTMLNTV	Nixon 88, Johnson 92	47,45
	p24	298–2	LAI	DRFYKTLRA	T. Harrer	S
	gp41	589–2	LAI	ERYLKDQQL	Johnson 92	45
HLA-B18						
	nef	135–2	LAI	YPLTFGWCY	Culmann 91, Culmann 94	33, 41
HLA-B27				2 C R K R	Jardetzky 91	48
	p24	263–2	LAI	KRWIILGLNK	Nixon 88 b, Buseyne 93	49, 50
	gp41	590–2	LAI	RYLKDQQL	Shankar 95	36
	gp41	791–2	LAI	GRRGWEALKY	Liebermann 92	35
	nef	73–2	LAI	QVPLRPMTYK	B. Culmann	PC
	nef	134–2	LAI	RYPLTFGW	B. Culmann	PC
HLA-B35				2 C P Y S	Hill 92	42
	p17	124–2	LAI	NSSKVSQNY	Rowland-Jones 95	51
	p24	254–2	LAI	PPIPVGDIY	Rowland-Jones 95	51
	RT	342–2	LAI	HPDIVIYQY	Rowland-Jones 95	51
	gp120	42–2	LAI	VPVWKEATTTL	B. Wilkens	U
	gp41	611–2	LAI	TAVPWNASW	Johnson 94 b	30
	nef	74–2	LAI	VPLRPMTY	Culmann 91, Culmann 94	33, 41
	HIV-2 gag	245–2	HIV-2	NPVPVGNIY	Rowland-Jones 95	51
HLA-B37						
	nef	120–2	LAI	YFPDWQNYT	Culmann 91	33, PC
HLA-B39						
	p24	193–2	LAI	GHQAAMQML	I. Kurane, K. West	PC
HLA-B53				2 C P Y F W	Hill 92	42
	HIV-2 gag	173–2	HIV-2	TPYDINQML	Gotch 93	52
HLA-B55						
	gp120	42–2	LAI	VPVWKEATTT	Shankar 95	36
HLA-B57						
	p24	240–2	LAI	TSTLQEQIGW	P. Goulder	U
	nef	116–2	LAI	HTQGYFPDWQ	Culmann 91	33, PC
	nef	120–2	LAI	YFPDWQNYT	Culmann 91	33, PC

Table 1 (cont.) Best Defined HIV CTL Epitopes

HLA	Protein	AA	Isolate	Sequence	Reference	Ref No*
HLA-Bw62						
	p17	20–2	LAI	RLRPGGKKKY	Johnson 91	23
	p24	268–2	LAI	LGLNKIVRMY	Johnson 91	23
	RT	476–2	LAI	ILKEPVHGVY	Johnson 91	23
	nef	84–2	LAI	AVDLSHFL	Culmann 94	41
	nef	117–2	LAI	TQGYFPDWQNY	B. Culmann	PC
HLA-Cw4				2 C	Falk 94	53
				Y F		
				P L		
				F M		
	gp120	380–2	LAI	SFNCGGEFF	Johnson 93	54

*In Ref No column the following abbreviations apply:
U = unpublished
PC = personal communication
IP = in press in *AIDS*
S = submitted

IV. Emerging characteristics of HIV-1 CTL epitopes

Although the list of HIV derived CTL epitopes is far from being complete, a few features already emerge. There are far fewer epitopes identified in the natural immune response to HIV than the prediction based on binding motifs would suggest, which may be due to a variety of factors. One is that the number of predicted binding candidates depends strongly on the accuracy of the binding motif. Thus, a well defined binding motif reduces the number of peptides that seem to have binding capacity andwhichthereforehaveto betested forbinding; and italsoalsoenhances the chancesofthe remaining peptides to actually bind to the class I molecule. It is also likely that not every binding peptide will be generated in the infected cell, or that there are other constraints on processing or binding to the newly synthesized class I molecule in the endoplasmatic reticulum such that the peptides are nor presented at the cell surface (i.e. flanking sequences, ref. 10). Furthermore, sequences of peptides very similar to self sequences may not be targeted by precursor cells within the self-tolerant CTL population, which may additionally reduce the number of possible CTL epitopes.

The expanding list of CTL epitopes also provides a perspective on regions which appear to be highlyimmunogenicforCTLresponses. Oneexampleisa10a.a. sequenceingp41(589–598),inwhich three different class I molecules (HLA A24, B8 and B14) present peptides containing CTL epitopes [11,12]. Another region of such overlapping CTL epitopes is the RT region 476–485, containing epitopes restricted by HLA-A2 and -Bw62, and the region 73–82 in the Nef protein, which contains epitopes restricted by HLA-A3, A11 and B35 [13]. Recently, several epitopes were defined within a short region at the C-terminal end of the nef-protein (183–196, HLA-A2, A52, B35; [14]). It is not known how this accumulation of CTL epitopes within certain regions occurs. In non-immunogenic regions, it is possible that some parts of the proteins are not accessible for the processing machinery (proteasomes) of the infected cell, and thus no CTL epitopes are generated from those areas, perhaps due to sequence peculiarity or to interactions of the viral proteins with host structures or viral products. In contrast, highly immunogenic areas may have advantageous flanking regions that allow efficient processing of these regions [10].

Sequencevariationhasbeenobservedinallthepeptidesinthetable. Althoughthereare differences inthe degreeofconservation, no epitope is derivedfrom anabsolutely conserved region, althoughsome

epitopes are localized in highly functional regions. For example, the gp120 peptide 424–432 (restricted by HLA-A32) contains sequences involved in CD4 binding and the peptide Gag p17/18–26 (HLA-3.1 restricted)is located within a region required for nuclear localization, which is important in infection of non-dividing cells [15]. Peptide RT 346–354 (HLA-A2 restricted) contains part of the active catalytic site of the HIV polymerase [16]. All these three peptides show only moderate intrinsic variability and may thus represent relatively conserved epitopes.

V. Conclusions

The investigation of the CTL response to HIV-1 has led to the most extensive characterization of the spectrum of class I restricted CTL epitopes of any viral infection studied this far. However, numerous questions remain that are of particularly acute interest. The reasons for the observed decline in CTL activity with disease progression remains undefined, and issues regarding immune escape and clonal exhaustion need further investigation [1,17]. The relationship between CTL pressure and in vivo sequence variation needs to be defined, as does the potential for altered peptide ligands to influence disease progression [18–20]. The relative contributions of specificity and breadth of the response to in vivo efficacy remain controversial, with some studies suggesting that a broadly directed response is favorable whereas others suggest that a narrowly directed response to a dominant epitope is advantageous [21,22]. Especially with regard to the development of a CTL vaccine, this controversy and the demonstration of the protective role of CTL in HIV infection have to be resolved. To address these issues it is essential to characterize the CTL response in its epitope-diversity as well as in its T cell receptor usage, and to compare these findings with variations in the viral genome. To this end, all available information about CTL epitopes, their variability and the responding T cell populations has to be verified and collected to offer a reliable basis to understand the cellular immunologic events occurringafter HIVinfection. We thus planto update this list periodically and would appreciate further information from any investigator who can contribute new data to keep this list current.

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